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DETERMINATION OF TOXIC METALS (Ni, Pb, Cr & Cd) IN SELECTED BRANDS OF CHOCOLATES AND CANDIES MARKETED IN BAHIR DAR CITY, ETHIOPIA

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ABSTRACT

Chocolates and candies are among sweet food items consumed primarily by children. In the present study, four toxic metals; cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) were determined in different brands of chocolate and candy samples available in local markets of Bahir Dar city, Ethiopia. Three chocolate and three candy brands were randomly selected. The concentrations of toxic metals under study were determined by inductively coupled plasma optical emission spectrometry (ICP−OES) following the conventional wet-acid digestion on an electric hot plate. The concentrations of Cd, Ni, Cr and Pb were analyzed in different brands of chocolate and candy samples. The concentration of Ni ranged from 0.95 ± 0.022 mg/Kg to 3.56 ± 0.055 mg/Kg for chocolate samples was the highest among all other toxic metals determined. Lead was absent in two brands of chocolate and one brand of candy. Cadmium was ranged from 0.067 ± 0.001mg/Kg to 0.1 ± 0.002 mg/Kg in chocolates and from not detected to 0.15 ± 0.0005mg/Kg in candy samples. Excel computer system, one-way analysis of variance (ANOVA) and origin software were used for data analysis. Except for Cr in candy samples, the ANOVA result showed that there is no significance difference in the mean concentration of toxic metals ($P > 0.05$). The results were compared with the provisional maximum tolerable daily intake (PMTDI) which is set by joint FAO/WHO expert committee on food additives (JECFA). Except cadmium, the other toxic metals were found to be above the limit.

Keywords: Toxic metals, Chocolates, Candies, ICP−OES, wet−acid digestion

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1. INTRODUCTION

Chocolate is an ingredient usually used in the preparation of several foods (cakes, cookies, breads, ice creams, etc.), which are consumed mainly by children. This indicated that the mineral content of this ingredient has a tremendous value for nutritional control of the foods. Nevertheless, the determination of toxic metals like Ni, Cd, Pb and Cr in such samples requires a difficult step of digestion because its matrix consists of high contents of organic compounds [1].

Chocolate is a very good nutritious energy source, with a fast metabolism and good digestibility. The presence of cocoa, milk and sugar in its composition make it the proper source of proteins, carbohydrates, fats, minerals and vitamins. Other main ingredients of chocolates are cocoa butter, hydrogenated vegetable oil and vegetable fats. [2]. It is therefore necessary to monitor human exposure to toxic metals present in the diet [2, 3]. Candies and chocolates are associated with joy and pleasure, potentially being stimulant, relaxant, and antidepressant. Different types of candies and chocolates are available in the national and international market [3, 4].

Recently, there is an increasing concern about the quality of imported foods and food related products in several parts of the world [4] particularly from overseas due to the large size of shipments, the variety of food imported and the large numbers of potential contaminants. Importantly, food products such as candies that are likely to be eaten frequently by children are covered by colorful packages, which can induce heavy metals such as Pb and Cr from the printed surface to the food contact surface [5].

Some heavy metals such as Pb, Cd and Ni may be toxic for human body if excessive amounts are taken [6]. Nickel occurs naturally more in vegetables than in animal flesh. However, its toxicity in humans is not a very common occurrence because the intestinal absorption of nickel is very low [6, 7].

Toxicity of cadmium is known by toxicity symptoms like gastrointestinal pains, nausea, respiratory distress, diarrhea, impaired reproductively, kidney damage and hypertension [8]. Cadmium is a heavy metal found as an environmental contaminant, both through natural occurrence and from industrial and agricultural sources.

Lead contamination in chocolates and candies is a very old problem that has evolved with time. Since the middle of the 19^{th} century various measures including regulations and public education were implemented to minimize the contamination of chocolates and candies from such sources [9]. Nowadays, industrial activities dominate the global flux of lead on the environment [8, 9] and have become predominant sources of lead in many food items, including candies [10].

Different complex matrices of the analytical sample required prior mineralization for most analytical methods, and this method is critical in the complete analytical procedure for the determination of metal concentration. Analysis of metals in food samples has conventionally been performed by atomic absorption spectrometry [11, 12]. Sample digestion techniques, such as microwave and conventional acid digestion

method (CDM) for toxic metals determination have been used widely for the dissolution of target elemental analysts. These digestion techniques however require the use of concentrated mineral acids and high temperatures whereas high pressures are required in the case of microwave applications [13].

Chocolates and candies are favorite food items for children who are the most susceptible age groups to toxic metal intake from such types of food items. Most of them, in Bahir Dar city, are sold at roadside shops, supermarkets, near schools, university campus shops and other small kiosks. Further, they are packaged by colorful wrappers, which might result in contamination of candies and chocolates by toxic metals.

Due to the contamination of toxic metals and other hazardous substances, candies and chocolates may pose human health risks. The current study is aimed to determine the concentration of Ni, Cd, Pb, and Cr in different brands of candies and chocolates available in the local markets of Bahir Dar city.

2. MATERIALS AND METHODS

2.1 Chemicals

All the chemicals and reagents used for the laboratory work were of analytical grade. Distilled water has been used throughout the laboratory work for sample preparation, dilution and rinsing each apparatus before working with it. HNO_3 (65%, Spectrosol, BDH, UK) and H_2O_2 (30%, Blulux, Laboratories, PLTD, India) were used for sample digestion. Stock standard solutions containing 1000 mg/L of the metals Ni, Cd, Pb and Cr were prepared from $NiCl_2.6H_2O$,$Cd(NO_3)_2$, $Pb(NO_3)_2$, and $Cr_2(SO_4)_3$, respectively of which all were from Sigma Aldrich. Working solutions were prepared from the stock standard solutions of metals by appropriate dilution of the intermediate solution (10 mg/L).

2.2 Sample preparation and digestion of samples

The samples collected from the local markets in Bahir Dar city were dried using microwave oven (GALLENKAP) for about three hours at 70^0C operating temperature and then ground into pieces. Around 20.00 g of each sample was kept until digestion. From this mass of sample, about 1.50 g of triplicate chocolate samples and 1.50 g of candy samples were measured and kept for digestion.

Conventional wet-acid digestion method was employed to digest the collected samples.10.0 ml mixture of HNO_3 (65%) /H_2O_2 (30%) (v/v) (1:1 ratio) was used by maintaining the temperature at 80 ^{0}Cfor 2−3 hours on the hot plate. After cooling the resulted clear solution for about 10 minutes, 15 ml of distilled water was added and mixed. Then, each replicate sample was transferred into 50ml volumetric flasks using a funnel and Whatman No.41 filter paper. The resulted solution was diluted to 50 ml with distilled water.

3. RESULTS AND DISCUSSION

3.1 Instrument calibration: linearity of calibration curves

The quality of data resulted from ICP−OES measurements are affected by sample digestion, calibration and standard solution preparation procedures [14]. In this study, the instrument was calibrated by using five series of working standard solutions prepared from intermediate solutions for each metal under determination. For the determination of Ni, Pb and Cr the range of concentration for standard solutions was from 0.001 mg/L – 2.401 mg/L. For Cd determination, it was in the range of 0.001 mg/L – 1.296 mg/L.

The intensity of each standard solution for each toxic metal was measured by using ICP−OES and the calibration curves were plotted. The squared correlation coefficient (R^2) values of calibration curves for Ni, Pb, Cr and Cd were 0.9973, 0.9964, 0.9992 and 0.9947 respectively. These high square correlation coefficient values indicate the linearity of the calibration curves.

3.2 Field Emission Scanning Electron Microscopy (FESEM)

To verify the precision of the method, each sample was measured three times. The percentage relative standard deviations (% RSD) of measurements were calculated. Based on this, the % RSD value of Ni, Pb, Cr and Cd for the analysis of chocolate was found to be 2.08 %, 11.68%, 5.27 % and 11.39%, respectively.

In order to evaluate the accuracy of the method, the recovery tests were studied for four toxic metals. Percentage recovery of the spiked concentrations for Ni, Pb, Cr and Cd in chocolate and candy samples was calculated by using the following equation.

$$\%RC = 100\ x\left(\frac{SPS - S}{SP}\right)$$

Where % RC = percentage recovery (in %)

SPS = amount found in spiked sample (in mg/L)

S = amount found in unspiked sample (in mg/L)

SP = amount added as a spike (in mg/L)

Percentage recovery of the spiked metals ranged from 89.6 % to 97.2 % for chocolate samples and from 87.0 % to 99.4 % for candy samples.

TABLE 1: Percentage recovery of spiked concentrations

Samples	Toxic metals	Concentration before addition of a spike (mg/L)	Amount of Spike added (mg/L)	Concentration of the spiked solution (mg/L)	% Recovery
Chocolate	Ni	0.084	0.200	0.2638	89.9
	Pb	0.072	0.200	0.2632	95.6
	Cr	0.134	0.200	0.3132	89.6
	Cd	0.001	0.200	0.1954	97.2

Candy	Ni	0.229	0.200	0.4278	99.4
	Pb	0.049	0.200	0.2230	87.0
	Cr	0.279	0.200	0.4758	98.4
	Cd	0.018	0.200	0. 2044	93.2

3.3 The limit of detection (LOD) and quantification (LOQ)

The LOD is defined as the lowest concentration of an analyte in a sample that can be detected. The LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the analytical conditions of the method. The LOD values were calculated by using an equation, LOD= $\frac{3SD}{m}$, where SD is for standard deviation and m is for slope of the blank in the calibration curve of each toxic metal. Similarly, LOQ values were calculated by the equation, LOQ= $\frac{10SD}{m}$.

Based on the above equations, the calculated LOD values for Ni, Pb, Cr and Cd were 0.846, 0.077, 0.055 and 0.108 mg/L, respectively. The calculated LOQ values were 2.807, 0.256, 0.184 and 0.363 mg/L, respectively.

3.4 Concentration of toxic metals in different brands of chocolate and candy samples

The concentration of four toxic metals (Ni, Pb, Cr and Cd) was determined by ICP−OES in different brands of chocolates and candies. Replicate solutions of each sample were measured. The concentrations were calculated using the mean of each value with standardization of the instrument by using the standard solutions and blank reagent. Toxic metal concentrations were based on the samples' dry weight and the mean results are the average values of the three replicates. The results indicate that the mean concentration of Ni, and Cr in chocolate samples was greater than that of their mean concentration in candy samples. On the other hand, the mean concentration of Pb and Cd in candy samples was greater.

Concentrations in mg/Kg of each toxic metal in the digested samples were also calculated using the following expression.

$$[M] = \frac{C}{W} \times V$$

[M] = total metal concentration (in mg/Kg)
C = concentration of the metal in the digested samples (in mg/L)
W = weight/mass/ of the digested sample (in g)
V = final volume of the digested sample solution (ml)

TABLE2: Concentrations (mean ± SD) of toxic metals determine different brands of chocolates and candies

Sample	Brand Name	Concentration (mg/Kg)			
		Ni	Pb	Cr	Cd
Chocolate	Galaxy(ChB−1)	0.95 ± *0.022*	0.23 ± *0.060*	ND	0.067 ± *0.001*
	Take (ChB−2)	3.56 ± *0.055*	ND	2.1 ± *0.050*	0.1 ± *0.002*
	Combo (ChB−3)	1.52 ± *0.010*	ND	2.13 ± *0.017*	0.1 ± *0.001*
Candy	Toppy (CB−1)	ND	ND	ND	ND
	Lollipop (CB−2)	2.94 ± 0.107	2.29 ± *0.022*	0.28 ± 0.034	0.15 ± *0.005*
	Desta (CB−3)	1.4 ± 0.030	0.91 ± 0.070	0.23 ± 0.035	0.12 ± 0.016

*ND = Not detected

3.4.1 Comparison of the average toxic metal concentrations among the different brands of chocolates and candies

From the three chocolate brands, only ChB−1 was found to be free from Cr. Pb was absent in the two brands of chocolate samples. On the other hand, Ni and Cd were found in all chocolate brands. In the case of candy samples, except the first brand of candy (CB−1), the rest were found to contain toxic metals.

Chocolate samples: From all the determined TMs, Ni was found in the greatest concentration. Its concentration ranged from 0.95 mg/Kg for ChB−1 to 3.56 mg/Kg for ChB−2. The least determined toxic metal was Cd whose concentration range is from 0.067 mg/Kg to 0.10 mg/Kg. Pb was found in a concentration of 0.23 mg/Kg only in ChB−1 chocolate brand. It was not detected in the rest of two chocolate brands. On the other hand, the content of Cr concentration ranged from ND to 2.13 mg/Kg. In general, Ni and Cd were detected in all chocolate brands whereas Pb is least detected. Figure 1 shows the comparison of concentration of TMs in different brands of chocolates.

Candy samples: Three different brands of candies were subjected to toxic metal determination. None of the toxic metals were detected in candy brand CB−1. The rest two brands of candies were found to contain toxic metals. The concentration of toxic metals in both CB−2 and CB−3 was in the order of Ni > Pb> Cr > Cd. The concentration of Ni ranged from ND (in CB−1) to 2.94 mg/Kg (in CB−2). The concentration of Pb, Cr and Cd was found in the range of ND to 2.29 mg/Kg, 0.28 mg/Kg and 0.15 mg/Kg, respectively, for the three candy samples. Figure 2 shows the comparison of concentration of toxic metals in the two candy brands.

From the toxic metals, in all chocolate and candy brands, the concentration of Cd was found to be relatively least than any other toxic metal determined. Its concentration is somewhat varied in candy samples. However, it has comparable concentrations in

chocolate samples. Its content was in the range of 0.067 mg/Kg to 0.10 mg/Kg and from ND to 0.15 mg/Kg in chocolate and candy samples respectively. In the case of Pb, it was least detected in chocolate samples even if its concentration is relatively high, i.e., 0.23 mg/Kg for chocolate samples and in the range of ND to 2.29 mg/Kg for candy samples.

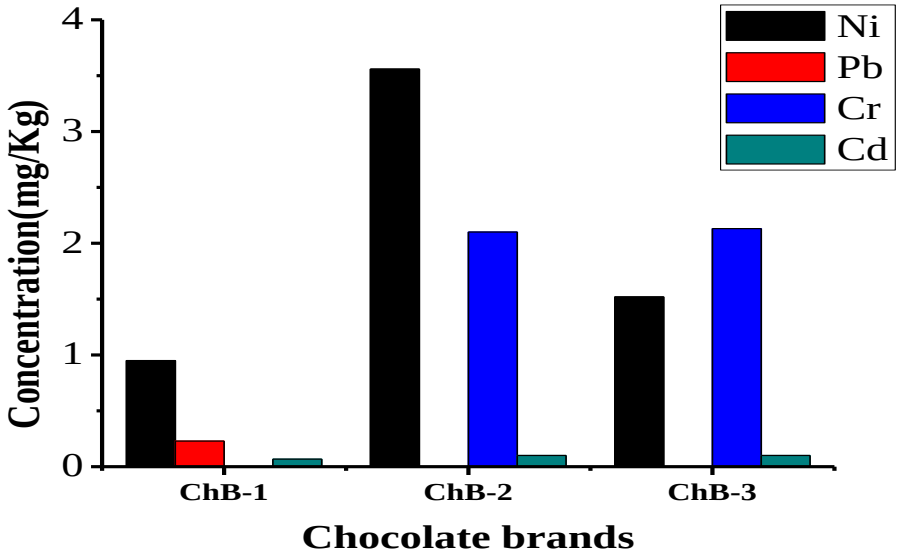


Figure1: Mean concentration of TMs in the three brands of chocolates.

In general, the concentration of toxic metals in different brands of chocolates and candies marketed in Bahir Dar city is relatively higher and its amount is significant. This high concentration of toxic metals in such types of food items is expected because of the ingredients and packaging wrappers used by the manufacturers. Relatively, chocolate samples were found to contain higher concentration of toxic metals. The high concentration of Ni may be due to its usage as a catalyst during manufacturing process of chocolate, i.e., when hardening is done by hydrogenation of unsaturated fats.

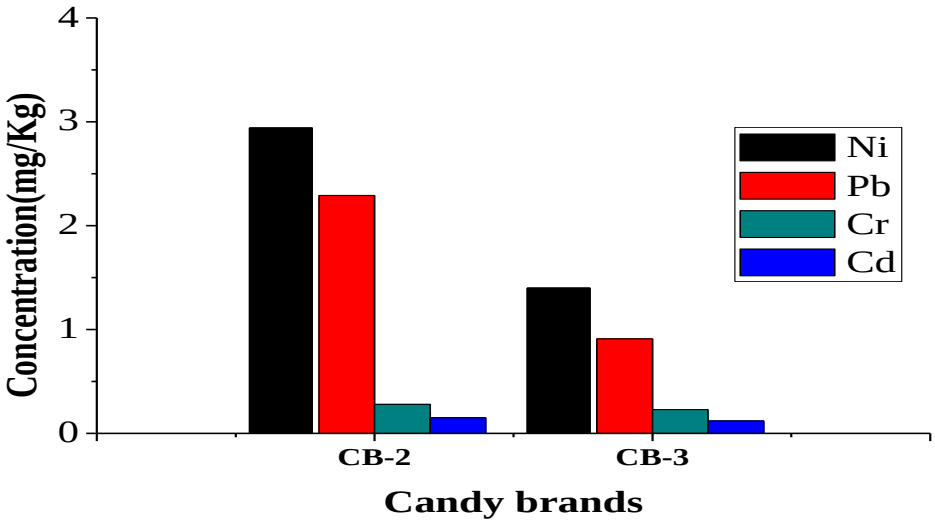


Figure 2: Mean concentration of TMs in the two brands of candies.

In previous researches, the concentration of Ni was reported in the range of 0.15 mg/Kg −3.55mg/Kg with a mean of 0.88mg/Kg for cocoa, milk and sugar-based chocolates [15]. In Pakistan, a similar research conducted to determine the concentration of toxic metals showed that the concentration of Ni ranged from 1.45 mg/Kg −4.33mg/Kg [10] of which the highest concentration was determined for cocoa-based chocolates as compared to milk and sugar-based chocolates.

The natural Ni content of various food items varies from 0 mg/Kg−10 mg/Kg [10]. The observed concentration for Ni in the present study is in agreement with this value, ranged from 0.95 −3.56 mg/Kg for chocolate samples and from ND−2.94 mg/Kg for candy samples. The highest Ni concentration (4.78 mg/Kg) was also obtained in the previous works done in Turkey [16]. Nickel's highest content in the previous works as well as in this present study is probably because of the high content of hydrogenated vegetable oil and cocoa butter which are the main ingredients used in the production of chocolates. Nielsen and Flyvholm (1983) suggested that nickel intakes in Denmark could reach over 900 μg/day by the consumption of certain foods having nickel composition, oatmeal, legumes (including soybeans), nuts, cocoa, and chocolate [17].

Research reports indicate that Pb contamination in chocolates and candies may be associated with atmospheric emission of lead gasoline as cocoa bean shells can absorb lead in a higher extent. The Industrial Revolution gave rise to an increase for lead in the environment and even bigger increase occurred around 1920 when leaded gasoline was introduced. Leaded gasoline is still not banned everywhere in the World, and still used in the developed countries. In areas where leaded gasoline is banned, the major exposure pathways of nonsmoking adults are from food [9]. Although the usage of leaded gasoline is not frequent and banned in most of the countries, there may be some

other sources of lead contamination like confectionery wrappers in chocolates and candies [16].

Another source of lead contamination could be packaging. Candy packaging has significantly higher levels of Pb (110−6394 mg/Kg) and Cr (137−1429 mg/Kg); particularly green and yellow- colored packaging has higher content of Pb which might be originated from the lead-based ink used to print the packaging [18].

Most of the metals analyzed in this study as well as in others are used in making alloys, which are used as containers to prepare candies and chocolates. Hence, utensils used during the preparation of candies and chocolates may cause metal contamination [18].

Cocoa beans can accumulate Cd naturally from the soil [19, 20]. As a result, its concentration could vary significantly in candies and chocolates. According to the FAO/WHO (2010) data, the level of Cd for chocolate brands ranges from 0.20 mg/Kg−2.00 mg/Kg [21]. The European Union also recommends the maximum of 0.30 mg/Kg Cd in chocolates containing ≥ 50% cocoa solid [22]. As compared to this, the present study showed lower level of Cd in chocolates as well as candies. However, it should be noted that the high intake of Cd could lead to distortion in calcium and the formation of kidney stones. It can also affect the skeletal and respiratory system [7, 23].

Chromium is another detected toxic metal in both of the samples. Except in ChB−1 and CB−1, it was detected in the rest of chocolate and candy brands selected for this study. A study conducted in Bosnia and Herzegovina showed the concentration of Cr in the range of ND−5.37 mg/Kg for chocolate samples of which the highest concentration belongs to cocoa-based chocolates [3]. In this present study, Cr was found to have an average concentration of 1.47 mg/Kg (ranged from ND−2.13 mg/Kg) for chocolate samples. For candy samples, its average concentration was 0.17 mg/Kg (ranged from ND−0.28 mg/Kg) which indicates that this much concentration of Cr in the present study is below the value determined in Bosnia and Herzegovina.

According to the research conducted in Serbia [21], the concentration of Cr in cocoa and milk chocolate samples was in the range of 0.12 mg/Kg−0.38 mg/Kg and ND−0.38 mg/Kg, respectively.

The presence of Pb and Cr in chocolate and candy samples can be linked with the colored packages (wrappers) of which pigments such as lead chromate ($PbCrO_4$), lead sulphate ($PbSO_4$) and lead oxide (PbO) could confer these colors [15]. These harmful pigments may intentionally be used in countries during manufacturing process. Accumulation of Pb in body tissues may cause acute toxicity by causing anemia, headache, fatigue, and peripheral nervous disorders [24]. Therefore, it is advised to minimize the excessive intake of food items that can contain Pb such as chocolates and candies.

What we understand from this is, parts of samples can be contaminated due to their packages. The result illustrates that some toxic metals could migrate from the packages when chocolate and candy surfaces are sticky, allowing the surface of the samples to adhere into the inner coverage of the package.

In general, the findings of this study indicate that toxic metals like Ni, Pb, Cr and Cd are found in food items like chocolates and candies. This higher concentration of toxic metals in candies and chocolates may be due to the raw materials such as cocoa beans, cocoa solids, hydrogenated vegetable oils and cocoa butter as well as their colorful packages.

3.4.2 Statistical analysis of variance (ANOVA)

The significance of variation in mean concentration of toxic metals between chocolate and candy samples was analyzed using one-way ANOVA. Based on the results, except for Cr, the mean concentration of toxic metals has not a significant difference between both chocolate and candy brands ($P > 0.05$) at the 95 % confidence interval. The variation of Cr within the two samples might arise from measurement errors.

4. CONCLUSION

In this study, three chocolate and three candy brands marketed in the city of Bahir Dar, Ethiopia were analyzed for their toxic metal content. The analysis was done after digesting the samples using conventional wet acid digestion method. Determination of the concentration of toxic metals was performed by using ICP−OES.

The results showed that all the toxic metals under study except Cr (in candies) and Cd found in chocolate and candy samples were generally higher than the JECFA limit. However, their concentration value agrees with most of other literatures. The concentration of Ni was found in the range of $(0.95 \pm 0.0215 - 3.56 \pm 0.0545)$ mg/Kg for chocolate samples and (ND -2.94 ± 0.107) mg/Kg for candy samples. Pb was found only in ChB−1 chocolate sample with concentration of 0.23 ± 0.06 mg/Kg and in two candy brands with a concentration of 0.91 ± 0.07 mg/Kg and 2.29 ± 0.022 mg/Kg.

Another toxic metal determined was Cr whose concentration ranged from ND -2.13 ± 0.0167 mg/Kg in chocolates and ND $- 0.28 \pm 0.034$ mg/Kg in candies. The Cd content in this study was below its values reported by other literatures as well as the JECFA provisional maximum tolerable daily intake limits. Its concentration ranged from 0.067 ± 0.001mg/Kg $- 0.1 \pm 0.0017$ mg/Kg and from ND-0.15 ± 0.0005 mg/Kg for chocolate and candy samples, respectively.

The reasons for the high levels of toxic metals in the samples could arise from the unsafe storage conditions or at any point in the production chain i.e., from the raw materials, processing, and packaging. The higher concentration of toxic metals in chocolates and candies may also be due to the raw materials such as cocoa beans, cocoa solids, hydrogenated vegetable oils and cocoa butter. The daily dietary intake of chocolates and candies must be reduced to keep the toxic metals within the prescribed limits set by JECFA. Hence, to reduce the dietary exposure, raw materials having lower content of these toxic metals should be used in the manufacturing process of chocolates

and candies. Furthermore, clear awareness should be created for the society about the existence of toxic metals in chocolates and candies marketed in Bahir Dar city.

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